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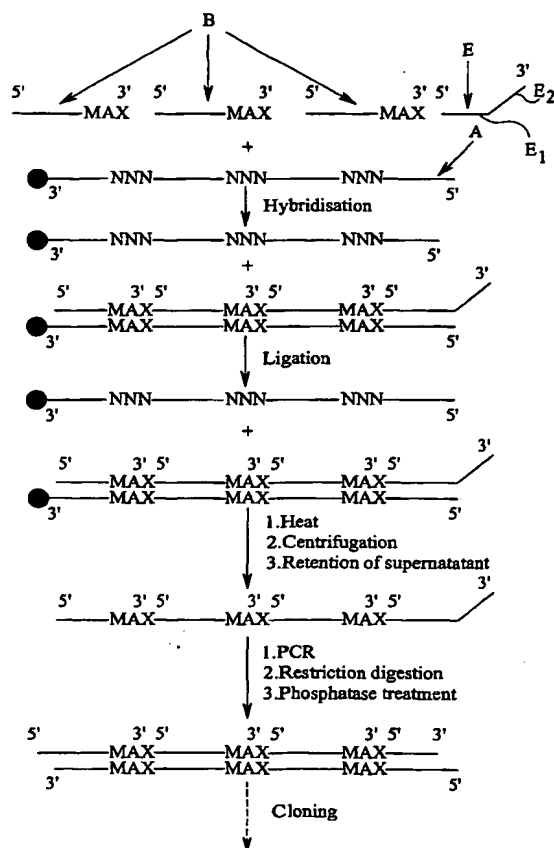
(GB). HUGHES, Marcus, Daniel [GB/GB]; 90 Hale-sowen Road, Cradley Heath, West Midlands, B64 5LU (GB). NAGEL, David, Andrew [GB/GB]; 41 Coventry Road, Kingsbury, Tamworth, B78 2LW (GB). ZHANG, Zhan-Ren [CN/GB]; 81 Greenoak Crescent, Birmingham, B30 2TD (GB). ASHRAF, Mohammed [GB/GB]; 42 Ellesmere Road, Birmingham, B8 1NG (GB). SUTHERLAND, Andrew, James [GB/GB]; Bracebridge House, 139 Chester Road South, Kidderminster, Worcestershire DY10 1XB (GB). SANTOS, Albert, Francis [GB/GB]; Llwynceilyn, Cefn Bychan, Pentyrch, Cardiff CF15 9PG (GB).

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[Continued on next page]

(54) Title: METHODS OF PRODUCING DNA AND PROTEIN LIBRARIES



(57) Abstract: The present invention provides a method of producing a DNA library comprising a plurality of DNA sequences of interest, where each DNA sequence of interest has at least two predetermined positions, with at each predetermined position a codon (MAX) selected from a defined group for that position, the codons within a group coding for different amino acids. The method comprising the steps of: - (i) contacting so as to effect hybridisation (a) template DNA (A) comprising said at least two predetermined positions, said template DNA being fully randomised at said at least two predetermined positions (NNN), (b) for each predetermined position, a selection oligonucleotide pool, each selection oligonucleotide (B) within each pool comprising a codon (MAX) selected from the defined group for that predetermined position, and (c) at least one additional oligonucleotide sequence (E) comprising a region (E<sub>2</sub>) which is non-hybridisable to the template DNA, (ii) ligating the hybridised DNA sequences (B, E), (iii) denaturing the product of step (ii) so as to give a mixed population of said template DNA (A) and said DNA sequences of interest, and (iv) selectively amplifying the DNA sequences of interest. The additional oligonucleotide sequence (E) of step (i) is selected such that after step (ii) the non-hybridisable region (E<sub>2</sub>) is located externally of the template DNA (A). The invention also provides protein and DNA libraries which can be produced by the method of the invention.



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## INTERNATIONAL SEARCH REPORT

In **national** Application No  
**PCT/GB 03/02573****A. CLASSIFICATION OF SUBJECT MATTER**  
**IPC 7 C12N15/10**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**Minimum documentation searched (classification system followed by classification symbols)  
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**MEDLINE, BIOSIS, EMBASE, EPO-Internal, WPI Data, PAJ****C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<b>T</b>	<b>HUGHES MARCUS D ET AL: "Removing the redundancy from randomised gene libraries."</b> <b>JOURNAL OF MOLECULAR BIOLOGY. ENGLAND 29 AUG 2003, vol. 331, no. 5, 29 August 2003 (2003-08-29), pages 973-979, XP002253425 ISSN: 0022-2836 the whole document</b>	<b>1-24</b>
<b>X</b>	<b>WO 00 15777 A (AMERSHAM PHARM BIOTECH UK LTD ;SANTOS ALBERT FRANCIS (GB); PALFREY) 23 March 2000 (2000-03-23) page 8, line 26 -page 10, line 2; claims 1-14</b>	<b>20,22-24</b>
<b>A</b>	<b>page 10, line 3 -page 19</b> <b>---</b> <b>-/-</b>	<b>1-19,21</b>

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

**4 September 2003**

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NEUNER P ET AL: "CODON-BASED MUTAGENESIS USING DIMER-PHOSPHORAMIDITES" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 26, no. 5, 1998, pages 1223-1227, XP001026093 ISSN: 0305-1048 the whole document</p>	1-24

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Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 0015777	A	23-03-2000	EP	1112355 A1	04-07-2001
			WO	0015777 A1	23-03-2000

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